

Murine pregnancy leads to reduced proliferation of maternal thymocytes and decreased thymic emigration

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Summary

During mammalian pregnancy the maternal thymus undergoes significant involution, and then recovers in size after birth. The mechanism behind this involution is not known, but it has been suggested that elevated levels of hormones during pregnancy induce the involution. We have recently shown that injection of 17 β -oestradiol into mice causes loss of early thymocyte precursors and inhibits proliferation of developing thymocytes. This suggests that elevated oestrogen in pregnancy may contribute to thymic involution. We have investigated this idea by examining the fate of thymocytes during mouse pregnancy in much greater detail than has been previously reported. Looking over a broad time-course, we find that pregnancy does not affect thymocyte precursor populations in the bone marrow, but induces a profound loss of early thymic progenitors in the thymus as early as day 12.5 of pregnancy. This loss is accompanied by decreased thymocyte proliferation, which returns to normal 2–4 days postpartum. No enhancement of apoptosis is detectable at any stage of pregnancy. We also find that there is a reduction in recent thymic emigrants after oestrogen treatment and at day 17.5 of pregnancy, suggesting that thymic involution during pregnancy influences the peripheral T-cell repertoire. The similarities between oestrogen-mediated involution and pregnancy-mediated involution suggest that oestrogen is a significant contributor to loss of thymocyte cellularity during pregnancy, and probably functions primarily by reducing thymocyte proliferation.

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Introduction

Although the thymus involutes with age, it still maintains a significant size and function during the childbearing years. During pregnancy, there is an acute decrease in the size of the maternal thymus in all mammalian species examined.¹ This is followed by a return to normal size shortly after birth. Evidence from mice suggests that the involution of the thymus that takes place during pregnancy may be important for normal fertility. Normal mice bearing a transplanted thymus that is resistant to involution (because of progesterone receptor deficiency) have an increased percentage of unimplanted and

resorbed fetuses compared to mice transplanted with thymuses that undergo normal atrophy during pregnancy.² Involution of the thymus may promote fetal survival by reducing the number of naive T cells entering the periphery, or by reducing the concentration of factors secreted by the thymus.

Despite the apparent importance of acute thymic involution during pregnancy, the mechanism for this process has not been defined. It is thought that hormones, which are elevated during pregnancy, are involved. Indeed, progesterone-receptor-deficient thymuses that have been transplanted into normal mice do not involute during pregnancy. High levels of oestrogen have also been been

Abbreviations: BrdU, bromodeoxyuridine; DN, double-negative; DP, double-positive; ETP, early thymic progenitor; FITC, fluorescein isothiocyanate; i.p., intraperitoneal; PBS, phosphate-buffered saline; PE, phycoerythrin; SP, single-positive; TCR, T-cell receptor.

implicated. Mice treated with high levels of oestrogen undergo acute thymic involution. Recently, we have defined specific aspects of thymocyte development that are influenced by oestrogen. We have demonstrated that levels of oestrogen similar to those obtained in the late stages of pregnancy do not increase thymocyte apoptosis, but instead reduce the proliferation of cells in the transition between the double-negative (DN) and double-positive (DP) stages. We also found that cells at the earliest stages of development in the thymus were significantly reduced.

These results suggest that both oestrogen and progesterone are involved in the involution of the thymus during pregnancy. However, the cellular events taking place in the thymus during pregnancy have not been evaluated in great detail. It is clear that in the later stages of gestation the DP cells in the maternal thymic cortex are severely depleted^{3,4} and one study evaluating thymocyte subsets during pregnancy showed an accumulation of a very immature thymocyte subpopulation, suggesting a block in T-cell development.⁴

In this study, we sought to define the cellular events in the thymus throughout murine pregnancy and the postpartum period. By comparing these results to our previous results obtained after oestradiol injection, we hoped to get a clearer picture of the role played by oestrogen during pregnancy-induced thymic involution. We found that thymic cellularity was reduced as early as day 12.5 of pregnancy, and that thymic involution occurred in the absence of detectable apoptosis. The size of the thymus rebounded in the days after delivery, and was restored to full size in 8–14 days. Our studies have revealed that pregnancy, similar to oestradiol treatment, inhibits proliferation of thymocytes at multiple stages of development, and leads to a profound depletion of the earliest progenitor populations in the thymus. In contrast to oestradiol treatment, the disappearance of these early progenitor populations in pregnancy is not preceded by a loss of thymic precursors in the bone marrow, suggesting that pregnancy directly affects the thymocytes or their supportive environment. We also find that during pregnancy the CD4 single-positive (SP) population contains an increased percentage of CD4⁺ CD25⁺ regulatory T cells, which are thought to protect the semi-allogeneic fetus. Finally, we demonstrate reduced thymic emigration at day 17.5 of pregnancy, showing directly that thymic involution during pregnancy influences the peripheral T-cell population.

Materials and methods

Mice

C57BL/6 mice were purchased from Charles River Laboratories (Raleigh, NC) or Jackson Laboratory (Bar Harbor, ME). Males were housed with females and the mice were

observed daily for formation of a vaginal plug. The day of plug observation was considered day 0.5 of pregnancy. All pups were removed from the mothers and killed within 24 hr of birth to prevent any variability as a result of lactation and weaning.^{4,5} All mice were used between 8 and 10 weeks of age. These studies were approved by the Emory University Institutional Animal Care and Use Committee.

Oestradiol injections

Age-matched male mice were injected intraperitoneally (i.p.) once daily with 200 µg β-oestradiol-17-valerate (Sigma-Aldrich, St Louis, MO) dissolved in ethanol and resuspended in sesame oil (Sigma) as previously established.⁶

Cell suspensions

Cell suspensions of thymus were prepared by gently grinding the organ between frosted glass slides (Fisher Scientific, Pittsburgh, PA) in phosphate-buffered saline (PBS; Sigma). Bone marrow was isolated from femurs. Cells were then resuspended in PBS and counted using a haemocytometer.

Flow cytometry

Antibodies and reagents used in flow cytometry were purchased from eBioscience [San Diego, CA; fluorescein isothiocyanate-conjugated anti-CD8 (anti-CD8-FITC), anti-CD3ε-FITC, anti-CD11c-FITC, anti-Ter119-FITC, anti-Gr-1-FITC, anti-NK1.1-FITC, anti-CD135-biotin, anti-CD3-biotin, anti-CD117-allophycocyanin (APC)], Caltag [Burlingame, CA; anti-CD11b-FITC, anti-CD8-tricolor, anti-CD45R-FITC, anti-T-cell receptor αβ (TCRαβ)-FITC, anti-TCRγδ-FITC, anti-CD4-APC, anti-CD8-APC, anti-CD25-phycoerythrin (PE), streptavidin-APC], BD Pharmingen [San Diego, CA; anti-CD25-FITC, anti-CD4-PE, anti-CD44-Cychrome, anti-CD4-FITC, anti-bromodeoxyuridine (BrdU) -FITC] or Leinco (St Louis, MO; anti-Sca-1-PE). Thymocytes and bone marrow were stained on ice with appropriate antibody dilutions in PBS containing 0.02% sodium azide and 0.5% bovine serum albumin. For analysis of apoptosis, thymocytes were stained with Annexin V-FITC (Caltag) according to the manufacturer's instructions. The lineage marker 'cocktail' for thymus includes anti-CD8, anti-CD3, anti-Ter119, anti-Gr-1, anti-CD11b, anti-NK1.1, anti-CD11c, anti-TCRαβ, anti-TCRγδ, and anti-CD45R. The lineage marker 'cocktail' for bone marrow includes anti-CD3, anti-CD4, anti-CD8, anti-CD11b, anti-CD45R, anti-Gr-1 and anti-Ter119. Cells were collected on a fluorescence-activated cell sorter (FACS) FACSCalibur (BD Immunocytometry Systems, San Jose, CA), and analysed using FLOWJO software (Tree Star, San Carlos, CA).

BrdU labelling

BrdU incorporation was detected with the BrdU flow kit (BD Pharmingen). Mice were injected i.p. with 1 mg BrdU and killed 5 hr later. Thymocytes were fixed in Cytofix/Cytoperm buffer, permeabilized in freezing media according to the manufacturer's instructions, and then reincubated in Cytofix/Cytoperm buffer. Cells were then treated with DNase to expose BrdU epitopes, and immunofluorescent staining was performed with anti-BrdU FITC and analysed by FACSCalibur.

Thymus cell export

A previously described technique⁷ was utilized for thymocyte labelling. Briefly, mice were anaesthetized, the upper chest was opened and the thymus lobes were exposed. Each thymus lobe was injected with 10 µl FITC (1 mg/ml) in PBS, which resulted in the labelling of 50–90% of all thymocytes. Mice were killed 24 hr later, and the recent thymic emigrants present in the spleen were identified by flow cytometry as live FITC⁺ cells. The number of emigrants was adjusted based on the percentage of thymocyte labelling in the corresponding thymus. Several control experiments establishing the validity of this technique have been previously reported.^{7–12}

Results

Thymic involution occurs during pregnancy in the absence of thymocyte apoptosis

The mechanism by which pregnancy-induced thymic atrophy occurs is currently unknown. By counting thymocytes at different time-points during gestation, we found that the thymus was undergoing involution by gestational day 12.5. We defined the day of vaginal plug formation as day 0.5. The thymus was reduced to $21 \pm 3\%$ the size of an age-matched non-pregnant thymus by gestational day 18.5, just before parturition. The thymus began to regenerate rapidly postpartum, reaching an average of 129.3×10^6 ($\pm 5.6 \times 10^6$) thymocytes 14 days after birth, compared to 115.3×10^6 ($\pm 5.6 \times 10^6$) in a non-pregnant control (Fig. 1a). Although our studies observed only syngeneic matings, our observations and previous studies have demonstrated a comparable decline in thymic mass between syngeneic and allogeneic breeding pairs.¹³ This indicated that thymic involution may not depend on the genotype of the fetus, but was more likely to be the result of increased levels of hormones associated with pregnancy.^{2,6}

Examining thymocyte subsets based on CD4 and CD8 expression at day 18.5 of pregnancy, when maximum involution is occurring, we found only minor changes in subset distribution (Fig. 1b). There was a slight decrease in the CD4/CD8 DP subset, while the other subsets

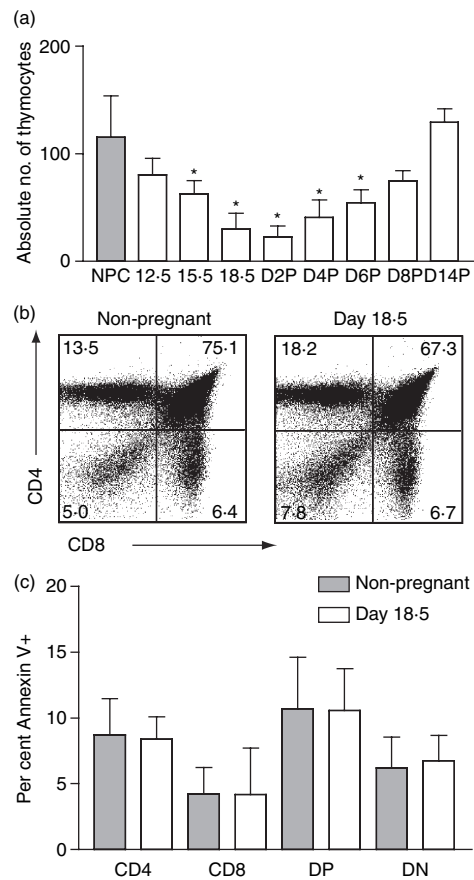


Figure 1. Pregnancy induces thymic atrophy in the absence of apoptosis. Thymus from non-pregnant control and pregnant female mice at various time-points throughout gestation were examined. (a) Absolute number of thymocytes was calculated using a haemocytometer. The data represent the mean of 3–48 mice \pm SD. Significance was determined by an unpaired two-tailed Student's *t*-test ($*P < 0.002$). (b) Thymocyte distribution at day 18.5 of gestation was examined by flow cytometric analysis of CD4 and CD8. The percentage of thymocyte subsets for a representative pair is shown. (c) The percentage of apoptotic cells within each thymic subset was determined using flow cytometric analysis of CD4, CD8 and annexin V. The data represent the mean of nine or 10 mice \pm SD.

remained relatively unchanged (Fig. 1b). This suggested that decreases in thymocyte number were fairly equal among the subpopulations. This could be caused by widespread apoptosis of thymocytes. To examine this, we utilized annexin V staining to determine if the thymocyte subsets are undergoing increased apoptosis at day 18.5 of pregnancy (Fig. 1c). We found no significant increase in apoptosis in any thymocyte subset at any time-point examined (data not shown). It remains possible that rapid clearance of apoptotic thymocytes precludes their detection. Our data suggest that the reversible thymic involution occurring during pregnancy was not accompanied by dramatic changes in thymocyte distribution or by an increase in apoptotic cells.

Loss of early thymic progenitors during pregnancy

T-cell precursors enter the thymus from the blood as CD4/CD8 DN cells. Development continues progressively through four DN developmental stages (ETP, DN2, DN3 and DN4), which are distinguished by their cell-surface expression of CD117 (c-Kit) and CD25. Lineage negative CD117⁺ CD25⁻ early thymic progenitors (ETP) progress through DN2 (c-Kit⁺ CD25⁺), DN3 (c-Kit⁻ CD25⁺) and DN4 (c-Kit⁻ CD25⁻) before up-regulating CD4 and CD8 to become DP thymocytes. Although the ETP population contains a small number of cells, changes in ETP number can have significant effects on overall thymus cellularity¹⁴ and we have observed a preferential loss of ETP in mice treated with oestradiol. Control of ETP number therefore represents a viable mechanism for pregnancy-induced thymic involution. Previous studies have demonstrated an accumulation of CD44⁺ CD25⁻ DN1 thymocytes during pregnancy, suggesting a block at the DN1 to DN2 transition.⁴ However, recent studies have shown that the DN1 population is quite heterogeneous, and true T-cell lineage precursors are lineage-marker-negative and express CD117, thus making the ETP population a better indicator of the most primitive thymocyte precursor population.¹⁵ We therefore examined DN thymocyte populations at various times during gestation using markers more specific for true T lineage cells. As seen in Fig. 2, the absolute number of the ETP, DN2, and DN3 populations was significantly reduced as early as day 12.5 of pregnancy and remained low as late as 6 days postpartum. The DN4 population was reduced with slightly slower kinetics, and changes mirrored the overall reduction in total thymic cellularity. The ETP and DN2 populations were slower to recover than the DN3 and DN4 populations; ETP were still significantly reduced 2 weeks postpartum. This suggests that pregnancy, like oestradiol treatment, has a profound effect on early thymic progenitors or the stromal cells that support them. Our analysis demonstrated that during pregnancy there was a profound block in T-cell development at the earliest developmental stages found in the thymus.

ETP are replenished by circulating blood cells derived from the bone marrow, and thymocyte precursors in the bone marrow are depleted by oestradiol treatment. The profound loss of ETP in the thymus over the long time-course of pregnancy led us to investigate the effects of pregnancy on bone marrow progenitors. On day 18.5 of pregnancy, there was a small but significant loss of total bone marrow cells, from 119×10^6 ($\pm 4 \times 10^6$) to 90×10^6 ($\pm 3 \times 10^6$) (Fig. 3a). This decrease however, was not accompanied by any significant alterations in the haematopoietic stem cell population, which we have defined as lineage negative, CD117⁺, and sca-1⁺ (LSK) (Fig. 3b). There was also no change in the further differentiated CD135⁺ (Flt3) LSK population, which is thought to contain the thymus-seeding cells^{16,17} (Fig. 3b). Thus,

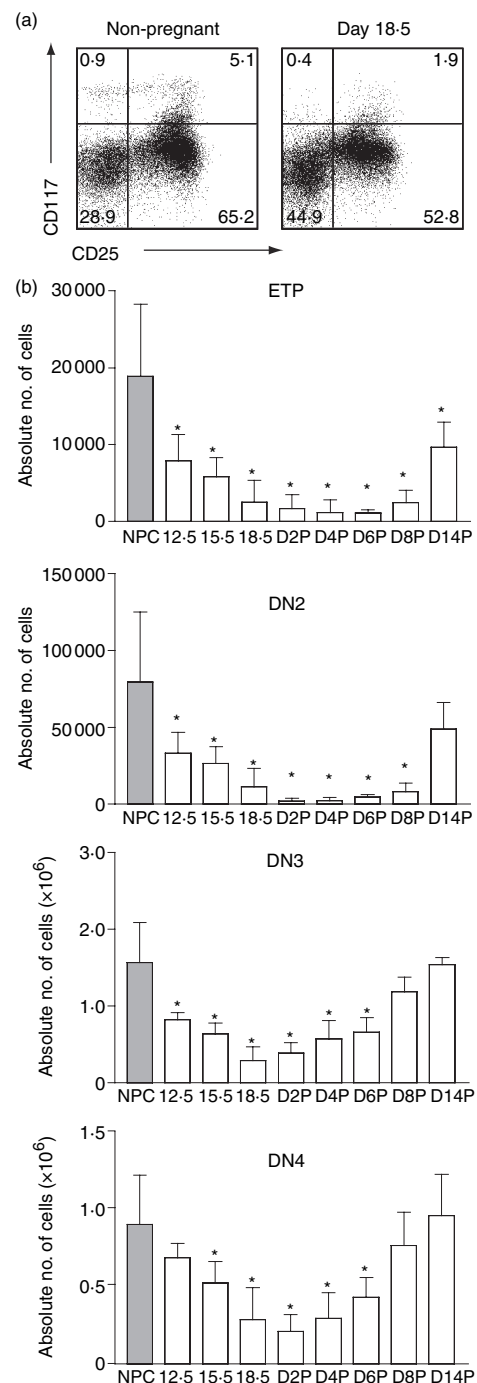


Figure 2. Pregnancy results in reduced numbers of thymic progenitors. Thymocytes from non-pregnant control mice and from pregnant females at various time-points throughout gestation were examined. (a) Dot plots for a representative age-matched pair of day 18.5 pregnant and non-pregnant control mice display staining for CD117 and CD25 after gating on lineage-negative thymocytes. The numbers in each quadrant indicate the percentage of lineage-negative cells. (b) The absolute number of each DN subset was compared to appropriate non-pregnant controls. The data represent the mean of 3–50 mice \pm SD. Significance was determined by an unpaired two-tailed Student's *t*-test (**P* < 0.03).

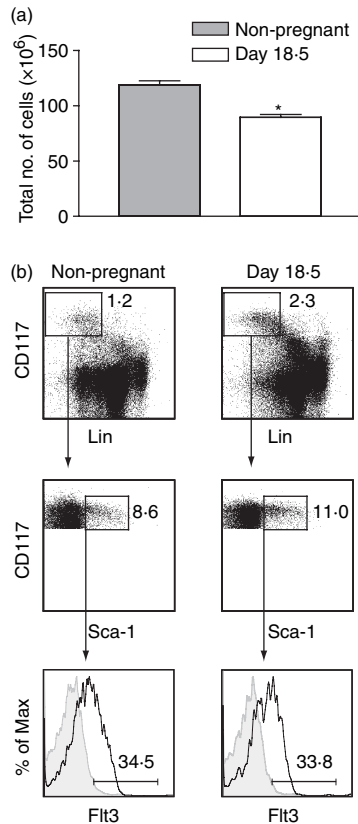


Figure 3. Decrease in total bone marrow cells during pregnancy. Bone marrow from female C57BL/6 non-pregnant controls and day 18.5 pregnant females was harvested and examined by flow cytometry. (a) Absolute number of bone marrow cells was determined using a haemocytometer. The data represent the mean of 7–10 mice \pm SD. Significance was determined by an unpaired two-tailed Student's *t*-test (* $P < 0.0001$). (b) Representative staining of cells from age-matched non-pregnant control and day 18.5 pregnant female mice. Bone marrow was stained with lineage markers, CD117, Sca-1 and CD135 (Flt3) to delineate haematopoietic stem cells (Lin[−]CD117⁺ Sca-1⁺ CD135[−]) from thymus-seeding CD135⁺ LSK cells (Lin[−]CD117⁺ Sca-1⁺ CD135⁺).

pregnancy was not sufficient to deplete thymocyte precursors in the bone marrow. This result suggests that the loss of ETP in the thymus during pregnancy is the result of an intrathymic defect rather than the targeted loss of bone marrow progenitors, although the effect of pregnancy on homing of thymic progenitors to the thymus has yet to be examined.

Reduction in thymocyte proliferation during pregnancy

Our previous studies have shown that elevated levels of oestradiol can lead to inhibition of DN proliferation.⁶ To determine if the reduced size of the thymus in pregnancy could be the result of inhibition of thymocyte proliferation, we determined the uptake of BrdU in thymocytes

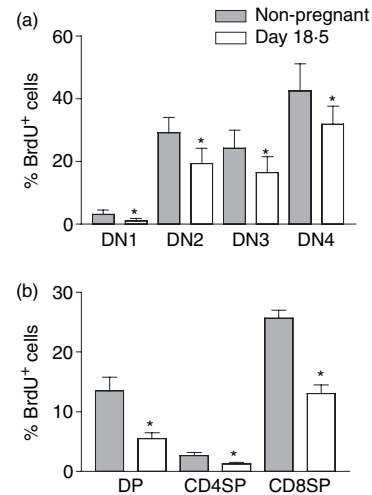


Figure 4. Inhibition of DN thymocyte proliferation during late stages of pregnancy. Non-pregnant control mice and pregnant female mice at day 18.5 of gestation were injected i.p. with 1 mg BrdU and killed 5 hr later. (a) Subsets were designated using CD3, CD4 and CD8 triple-negative thymocytes stained with CD44 and CD25. The percentage of BrdU⁺ thymocytes was determined by flow cytometry. The data represent the mean of seven or eight mice \pm SD. (b) Subsets were stained with BrdU, CD4 and CD8. The data represent the mean of three mice \pm SD. Significance was determined by an unpaired two-tailed Student's *t*-test (* $P < 0.02$).

from mice at day 18.5 of pregnancy. At this late stage of pregnancy there was a significant reduction in proliferation at all four DN stages (Fig. 4a). Interleukin-7 is thought to contribute to proliferation of DN2 thymocytes,¹⁸ while Notch and pre-TCR signalling are necessary for DN3 expansion.¹⁹ As we observed reduction in all populations, regardless of their proposed proliferative stimulus, this may indicate that elevated hormones during pregnancy suppress all proliferation in the thymus. In support of this, we also found a significant decrease in DP, CD4 SP and CD8 SP BrdU incorporation on day 18.5 (Fig. 4b). Previous studies have demonstrated a reduction in proliferation of mature T cells after oestradiol exposure, possibly as a result of decreases in cyclin A.^{20,21} Further studies will be necessary to determine the mechanism behind this inhibition, however, our current data suggest that inhibition of the proliferative capacity of all thymocyte subsets was a major contributor to thymic involution during pregnancy.

Late-stage pregnancy results in an increased proportion of CD4⁺ CD25⁺ thymocytes

Although fetal antigens could be perceived by a maternal immune system as foreign, several mechanisms have developed to protect the fetus from immune attack. One such mechanism that has been the subject of increasing interest involves regulatory T cells. Aluvihare *et al.*

showed that CD4⁺ CD25⁺ cells are necessary to maintain an allogeneic but not syngeneic pregnancy. They found an increased percentage of CD25⁺ CD4 cells in the spleen, blood and lymph nodes of mice during early stages of pregnancy.²² Increased levels of circulating regulatory T cells were also found during human pregnancy.²³ It is possible that altered thymopoiesis during pregnancy could contribute to the enhancement of regulatory T cells. As seen in Fig. 5(a), we observed an increase in the percentage of CD25⁺ CD4 SP cells in the thymus at day 18.5

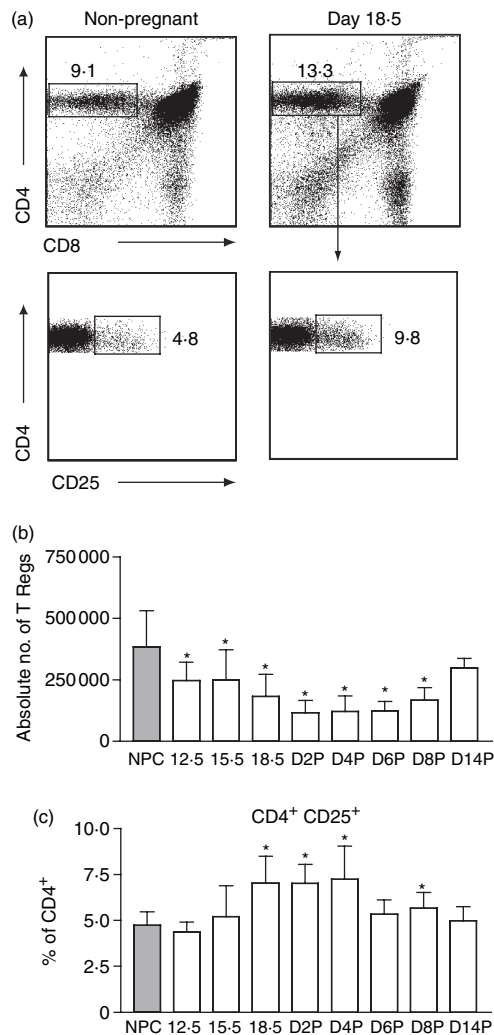


Figure 5. Pregnancy results in an increased percentage of CD4⁺ CD25⁺ thymocytes. Thymus from non-pregnant control and pregnant female mice at various time-points throughout gestation were stained with CD4, CD8 and CD25 and examined by flow cytometry. (a) Dot plots for a representative pair of age-matched day 18.5 pregnant mice and non-pregnant control mice are gated on live CD4 SP thymocytes. The absolute number (b) or percentage (c) of CD25⁺ CD4 SP thymocytes on various days was determined using flow cytometry. The data represent the mean of 3–47 mice \pm SD. Significance was determined by an unpaired two-tailed Student's *t*-test (**P* < 0.05).

gestation in our syngeneic model. Although the absolute number of CD25⁺ CD4 SP thymocytes decreased with pregnancy as a result of the profound decrease in thymus cellularity (Fig. 5b), the percentage of CD4⁺ thymocytes that were CD25⁺ increased at late stages of gestation (Fig. 5c). CD4⁺ SP thymocytes from non-pregnant control mice were $4.7 \pm 0.1\%$ CD25⁺, while those from day 18.5 of pregnancy were $7.0 \pm 0.5\%$ CD25⁺. This increased percentage declined gradually postpartum, and returned to average after 6–8 days. Thus, a change in the ratio of regulatory to non-regulatory T cells occurred in the thymus during pregnancy.

Pregnancy and elevated oestradiol levels result in decreased thymic output

The thymus undergoes significant involution during pregnancy, and Tibbetts *et al.* have shown that lack of thymic involution results in impaired fertility.² Thymic involution during pregnancy may be needed to reduce the production of new T cells. However, the effect that pregnancy has on thymic output has not been reported. To address this, we used intrathymic injections of FITC to label thymocytes on day 17.5 of pregnancy (Fig. 6a), and observed their appearance 24 hr later in the spleen. Previous studies have shown that intrathymic but not intravenous injections of FITC or direct injections into the mediastinal cavity resulted in substantial thymocyte labelling.^{7,8,10,11} Intrathymic injections of PBS did not result in any detectable thymic changes.^{8,9} Finally, FITC-labelled lymphocytes retain normal homing capabilities,¹² making this technique an ideal way to track recent thymic emigration. We found a significant decrease in recent thymic emigrants on day 17.5 of gestation when compared to non-pregnant controls (Fig. 6b). We found a similar reduction in thymic emigration using male mice that received five daily injections of oestradiol (Fig. 6c). This suggests that pregnancy or oestradiol-induced thymic involution can reduce thymic output, and perhaps alter the peripheral T-cell compartment to enhance fetal survival.

Discussion

In this study, our goal was to define the cellular changes taking place in the thymus during various stages of pregnancy. We have demonstrated that pregnancy-induced thymic involution occurs in the absence of detectable apoptosis and have revealed for the first time that thymic involution instead is accompanied by a loss of early T-cell progenitors in the thymus, and by reduced proliferation of CD4, CD8, DP and DN thymocyte subsets. We also showed an enrichment of CD4⁺ CD25⁺ regulatory T cells in the thymus during later stages of gestation, and we found markedly reduced T-cell emigration from the thymus at day 17.5 of pregnancy.

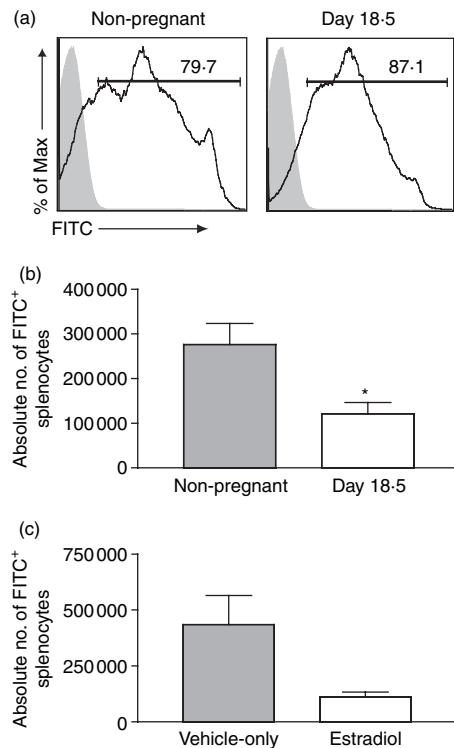


Figure 6. Oestradiol-treated and pregnant mice have reduced numbers of recent thymic emigrants. Mice were anaesthetized and 10 μ g FITC was injected directly into each thymic lobe. Spleens were harvested 24 hr later and examined for FITC⁺ cells by flow cytometry. The number of FITC⁺ splenocytes was then adjusted for labelling efficiency using the % FITC⁺ thymocytes from each respective animal. (a) Representative histograms of live thymocytes showing labelling efficiency. (b) Day 17.5 pregnant females and age-matched non-pregnant controls were compared. The data represent the mean of five or six mice \pm SD. Significance was determined by an unpaired two-tailed Student's *t*-test (**P* < 0.02). (c) Male C57BL/6 mice injected i.p. daily with vehicle only or 200 μ g oestradiol for 5 days were compared. The data represent the mean of two to four mice \pm SD.

Many have speculated that the nonspecific nature of pregnancy-induced thymic involution supports the involvement of maternal hormones. The oestrogens oestradiol and oestriol, as well as progesterone, increase significantly during gestation, and have been extensively investigated for their immunomodulatory effects. Studies have shown that progesterone receptor signalling is necessary for pregnancy-induced thymic involution.² Furthermore, continuous administration of progesterone can lead to a decrease in thymic size² although we find no changes in thymocyte subset distribution or proliferation when we inject progesterone into male mice (data not shown). Progesterone administration by itself has been found to have no effect on B-cell development, a process that is severely inhibited during pregnancy.²⁴ Some reports suggest a synergistic relationship between oestradiol and progesterone, whereby progesterone enhances the effects

of oestradiol.^{2,24} Future studies will be necessary to determine the exact role of progesterone in pregnancy-induced inhibition of B-cell and T-cell lymphopoiesis.

Unlike progesterone, the role of oestrogens in thymic involution has been well documented. Prolonged exposure to oestradiol or oestriol results in a significant loss of thymic cellularity, with a disproportionate loss of DP thymocytes.^{6,25–28} We have previously shown that this decrease is preceded by a loss of T-cell precursors in both the thymus and the bone marrow, in addition to reduced proliferation of DN3 and DN4 thymocytes.⁶ Neither pregnancy nor oestradiol-induced thymic atrophy is accompanied by an increase in apoptosis (Fig. 1c).⁶ Finally, both mechanisms of involution lead to decreased thymic output (Fig. 6). The presence of oestrogen receptors in both thymocytes and thymic stromal cells²⁹ in addition to the similarities between pregnancy and oestradiol-induced thymic involution suggest that oestrogens play a direct role in thymic atrophy in pregnant mice. Exogenous administration of oestradiol may serve as a useful model to study the immune alterations that occur during pregnancy.

In contrast to thymopoiesis, inhibition of B-cell lymphopoiesis during pregnancy has been well characterized. Medina *et al.* have shown a significant decrease in B-cell development in the bone marrow throughout gestation, which leads to a decrease in immature B cells in the periphery. Inhibition of B lymphopoiesis by pregnancy is at least in part the result of decreased mitotic activity of B-lineage lymphocytes, in addition to B-cell precursor depletion.⁵ These findings parallel the loss of DN proliferation and early thymic progenitors that we have demonstrated in the thymus of pregnant mice. This may suggest a common mechanism that suppresses both B and T lymphopoiesis. The profound inhibition of lymphopoiesis during pregnancy could be necessary to dampen the peripheral immune response to paternal antigens.

Many studies suggest that pregnancy results in an immune response that is skewed toward humoral immunity.^{30–32} Reduced production of naive T and B cells may contribute to this alteration of the maternal immune response. However, we find that despite reduced numbers of B-cell and T-cell progenitors at day 18.5 of pregnancy, the total number of nucleated cells in the spleen does not differ from age-matched non-pregnant controls (data not shown). We also find that the ratio of CD4 to CD8 T cells in the spleen does not change, although the number of naive versus memory T cells or T helper type 1 versus type 2 CD4 T cells has not been examined. In support of altered peripheral lymphocyte populations, we find a nearly 4.5-fold expansion of marginal zone B cells in the spleen during late stages of pregnancy (data not shown). This increase in marginal zone B cells is probably the result of the increased levels of circulating oestrogens associated with pregnancy.³³ Further studies will be neces-

sary to investigate pregnancy-induced changes among the various T-cell populations in the spleen and other peripheral immune sites.

Although both T-cell and B-cell development are dramatically inhibited during pregnancy, only modest changes are observed in the spleen. This raises the question as to the importance of reduced lymphopoiesis during gestation. In support of a critical role for thymic involution, Tibbetts *et al.* found reduced embryonic survival when the maternal thymus was resistant to involution.² There are several ways in which thymic involution could lead to improved fetal survival. First, we have demonstrated decreased thymic output at day 17.5 of pregnancy, which could lead to a reduction in naive T cells capable of recognizing paternal antigens. This may occur in the spleen, but could be more significant at other locations such as the fetal–maternal interface. We have also shown that CD25⁺ CD4⁺ regulatory T cells are less affected by thymic involution than CD25[−] CD4⁺ SP thymocytes. Enrichment for this subset may be critical for dampening the maternal immune response. Indeed, Aluvihare *et al.* have shown that CD25⁺ CD4⁺ regulatory T cells are necessary for an allogeneic pregnancy.²² It has also been demonstrated that pregnancy-induced thymic atrophy is accompanied by an expansion of intermediate TCR-expressing cells in the liver.³⁴ Increased levels of non-traditional T cells coupled with a decrease in thymic emigration may be an important factor in controlling the maternal immune response. Understanding the role of the thymus in fetal tolerance may provide further insight into maintaining a successful pregnancy.

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